OIE Guide 3:

International Reference Antibody Standards for Antibody Assays

1. Introduction

1.1. Purpose

This document provides guidelines for the preparation, validation and distribution of antibodies as International Reference Standards for antibody assays for infectious diseases of animals. In these guidelines, the term "Standards" refers to antibodies unless indicated otherwise. Such standard preparations are designated by the OIE as primary reference standards for use in conjunction with tests described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.*

1.2. Definitions

1.2.1. Standard Test Protocol

Standard Test Protocol refers to a validated, internationally accepted test procedure, often an 'OIE Prescribed Test for International Trade', which is described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.

1.2.2. International Reference Standard

The term International Reference Standard is synonymous with primary reference standard. It represents the standard by which all others are compared and calibrated.

1.2.3. Secondary and Working Standards

Secondary standards are prepared by direct comparison with the International Reference Standard, and should so far as is possible mimic the characteristics of the primary standard when used in the Standard Test Protocol. A Secondary Standard would typically be prepared by a National Reference Laboratory and be designated as the national or local standard.

Working standards may be synonymous with secondary standards, or they may be tertiary standards calibrated against the secondary standard. Working standards should be available in sufficient quantities for use by diagnostic laboratories to standardise routine daily testing.

1.3. Scope

International Reference Standards are necessary to ensure that a given antibody assay is capable of measuring antibody activity to a specified level of diagnostic sensitivity. Diagnostic sensitivity relates to the risk of a false negative reaction occurring in an antibody assay when in fact an animal is, or has been, infected. International Reference Standards are normally for use by international, national and other reference laboratories in calibrating standard assays and as templates for the production of secondary standards. The secondary or other working standard, and not the international standard, are to be used on a daily basis to standardise testing.

For a limited number of diseases, there has been international agreement on a system of 'International Units' of antibody activity. In such cases the International Reference Standards define the scale of such units. In the vast majority of animal diseases no such system exists, and assay systems, working standards, and test samples are defined relative to the International Reference Standards.

1.4. Approach

For most assays, three primary reference standards should be established: a strong positive. a weak positive and negative standard. These standards should be selected and characterised by a designated Reference Laboratory using an internationally accepted Standard Test Protocol and internationally accepted reagents.

The weak positive standard is critical for providing assurance of the diagnostic sensitivity of the test. For non-quantitative assays (e.g. immunodiffusion tests) the weak positive reference standard may be the only positive standard required.

For quantitative, non-titration assays such as indirect ELISA, the strong positive standard should define an arbitrary level of 100% positivity. The weak positive and negative standards should then be assigned a proportional percentage positivity corresponding to their reactivity when tested in the standard test protocol.

2. Selection of Materials for use as Standards

2.1. Types of material

The majority of International Reference Standards will be prepared from blood serum. This should be free from haemolysis and from excessive lipaemia. Antisera should where possible be produced in specific pathogen free or gnotobiotic animals of a species appropriate to the assay being standardised. Other materials, for example defatted milk, or monoclonal antibodies, may be used where appropriate to the assay being standardised.

2.2. Safety

The reference standards should be prepared so that they are free of infectious material. To facilitate shipment between countries it is recommended that the standards in the wet state be either treated with BEI (binary ethyleneimine) or irradiated at 25–30 kilogray (2.5–3.0 Mrad) and kept at –78°C. Irradiating freeze-dried samples is not recommended as the recommended dose may not be enough for complete pathogen inactivation. After treatment, samples should be submitted to appropriate innocuity tests to ensure that they are free from detectable live agents. Bovine sera should be from a BSE-free source.

2.3. Positive reference standards

Positive reference standards should be selected from animals which exhibit a typical humoral (i.e. antibody) immune response to the organism in question. Hyperimmune animals are not considered typical, and should be avoided if possible. The immune response may be elicited by experimental infection or by immunisation with vaccines. The timing after immunisation for collection of the material should be determined by the response of the animal as measured in the standard test protocol. This may vary according to the nature of the disease and the assay. Full details of the immunisation schedule and the nature of the immunogen must be provided so that secondary standards can be prepared by equivalent methods. The standards should be free from antibodies to organisms that might cross-react in the standard assay or information on this cross-reaction should be provided. The standard may be derived from a single animal or a pool of samples from a number of animals. Exceptionally, naturally infected animals may be used as the source of the standard where controlled immunisation or infection is not feasible.

2.4. Negative reference standards

Negative reference standards should be selected from animals that have never been exposed to, or vaccinated against, the organism in question. They should be free from antibodies to organisms which might cross-react in the standard assay. The negative standard may be derived from a single serum or a pool of sera.

3. Characteristics of International Reference Standards

3.1. Strong positive reference standard

For tests such as complement fixation, virus neutralisation or indirect ELISA, that demonstrate typical sigmoidal dose/response curves, the strong positive reference standard should exhibit an antibody activity which lies on the linear portion of the curve just below the plateau phase. In other tests, the strong positive reference standard should contain sufficient antibody to produce consistently the maximum reaction within the selected limits of the test, e.g. a clear cut line of identity in an immunodiffusion test or 100% inhibition in a competitive/inhibition ELISA.

3.2. Weak positive reference standard

The weak positive reference standard should exhibit an antibody activity which again lies on the linear portion of the curve just above the positive/negative threshold. The reaction produced should never be equivocal. In other tests, the weak positive reference standard should contain sufficient antibody to produce consistently the minimum detectable reaction, e.g. a weak but unequivocal line of identity in an immunodiffusion test. For competitive/inhibition assays which frequently show a sharp transition from positive to negative the selection of the weak positive standard can be particularly difficult. The same priniciples apply, in that the standard should give a consistent positive response, just above the positive/negative threshold, in the Standard Test Protocol.

3.3. Negative reference standard

This standard should always give a reaction below the positive/negative threshold in the Standard Test Protocol. The reaction produced should never be equivocal.

4. Preparation of Reference Standards

4.1. Constitution of the standards

Where possible, the positive reference standards should be prepared from materials showing the desired level of reactivity without further dilution. However in many cases it may be necessary for the Reference Laboratory to make a one time dilution of a positive serum in negative serum in order to achieve the desired level of reactivity as specified in (3) above. In such cases the weak positive reference standard may be derived from the same positive serum stock as the strong positive reference standard.

An International Reference Standard should not require any special manipulation (e.g. predilution) by the recipient laboratory prior to its use in the assay in question. The standard should be tested as would any field sample under routine diagnostic conditions (including any dilution steps which are a normal part of the assay procedure). This prevents the introduction of error or bias related to special handling or preparation. Therefore the amount of antibody activity in a positive reference standard should be within the accurate detection limits of the diagnostic test.

4.2. Stability and storage

All materials should be stored frozen or refrigerated pending evaluation. Repeated freeze–thaw cycles should be avoided. To ensure stability it is recommended that the final standard, after sample treatment to inactivate adventitious agents, be freeze-dried, and it would be advantageous to provide the sterile diluent for reconstitution of the material, along with the freeze-dried standard. Sealed glass ampoules, rather than rubber caps, are preferred for long-term storage. Freeze-dried stocks should be stored at 4°C, although short periods at ambient temperature (e.g. during shipment) should not be deleterious. The freeze-drying process may alter the biological quality of sera; storing the standards in cryotubes at –78°C is the recommended alternative solution.

After freeze-drying, several bottles of the standard should be reconstituted and re-evaluated. There should be no evidence of cross-reacting antibodies or other nonspecific factors which interfere with the interpretation of assay results. If there is a possibility of cross-reaction with closely related agents, this information should be indicated.

4.3. Batch control

The original reference material must begin as one single stock with enough to last at least 5 years. This can be kept frozen (preferably at –70°C or below) and a batch can be freeze-dried for a minimum 2-year supply (about 500 tests). For each batch, whether frozen or freeze-dried, batch references must be allocated and full quality control data maintained for each batch.

Each freeze-dried batch must be recalibrated. Each bottle or ampoule should contain 0.5–1 ml.

4.4. Labelling

The label should contain the following minimum information: OIE logo; OIE international reference standard for (disease) (test); specify if strong positive, weak positive or negative; the name of the Reference Laboratory; reconstitution method; and storage conditions. The space available on the label may prevent the inclusion of all these items; abbreviations may be used and some of the items may need to be put on the data sheet instead of on the label.

4.5. Data sheets

OIE Reference Laboratories issuing international reference standard sera should ensure that all aliquots are accompanied by an appropriate Data Sheet. It should be made clear to requesting laboratories that international reference standards are intended for use in the calibration of their own assay and for promotion of international harmonisation.

In order for a diagnostic laboratory to prepare a secondary reference standard for its own use, it will be necessary for the OIE Reference Laboratory to supply specific data on the selection and/or preparation of the primary reference standards. This is especially true when primary reference standards have been prepared by dilution of strong or hyperimmune positives in negative sera.

4.5.1. Data required

The datasheet should repeat all the information specified for the label (see 4.4). The following information must also be supplied in order to facilitate the selection and/or preparation of secondary reference standards which, as closely as possible, duplicate the primary reference standard.

i) Description of donor animal for positive and negative serum, including species, age, reproductive status and origin (i.e. natural production, specific pathogen free, gnotobiotic, etc.).

ii) Nature of antibody response, i.e. to natural infection, experimental infection, immunisation, etc.

iii) Details of organism used to elicit the immune response, i.e. source, strain, serotype, etc.

iv) Details of experimental infection or immunisation protocols, i.e. route, dose, immunisation schedules, method and time of sample collection etc.

v) Reference tests used to select positive and negative reference sera candidates and to characterise the antibody response, e.g. ELISA, agar gel immunodiffusion, virus neutralisation, etc.

vi) Sample of titration profiles of hyperimmune sera and criteria for selection of appropriate dilutions of defined activity.

vii) Presence of heterologous antibodies, if known, and tests used in detection.

viii) Details of any safety testing carried out on the materials

ix) A statement that the standard is for *in vitro* use only.

x) Description of sterilisation methods, including type of irradiation and dose and condition of sample at time of sterilisation (i.e. liquid, frozen, freeze-dried, etc.).

xi) Batch number and date of production.

xii) Recommended reconstitution (type of reconstituting fluid, and volume), handling and storage conditions.

xiii) Full contact address, fax, email of the Reference Laboratory as a source of further information.

5. Approval of Reference Standards by OIE

An International Reference Standard may not be issued under the name of OIE unless it has been endorsed by the OIE Standards Commission acting under authority of the OIE International Committee.

The full technical and statistical data on the evaluation of the candidate reference standards, together with the full data sheet information as specified above, should be submitted to OIE. The OIE Standards Commission will review the information. If the Standards Commission approves, the reference standard will be added to the list of International Reference Standards available. This list will be supplied to all OIE Members Countries on request, and may also be accessed on the OIE Web site (http:\\www.oie.int).

6. References

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WORLD HEALTH ORGANIZATION (2002). Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products. WHO Expert Committee on Biological Standardization. WHO, Geneva, Switzerland.

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